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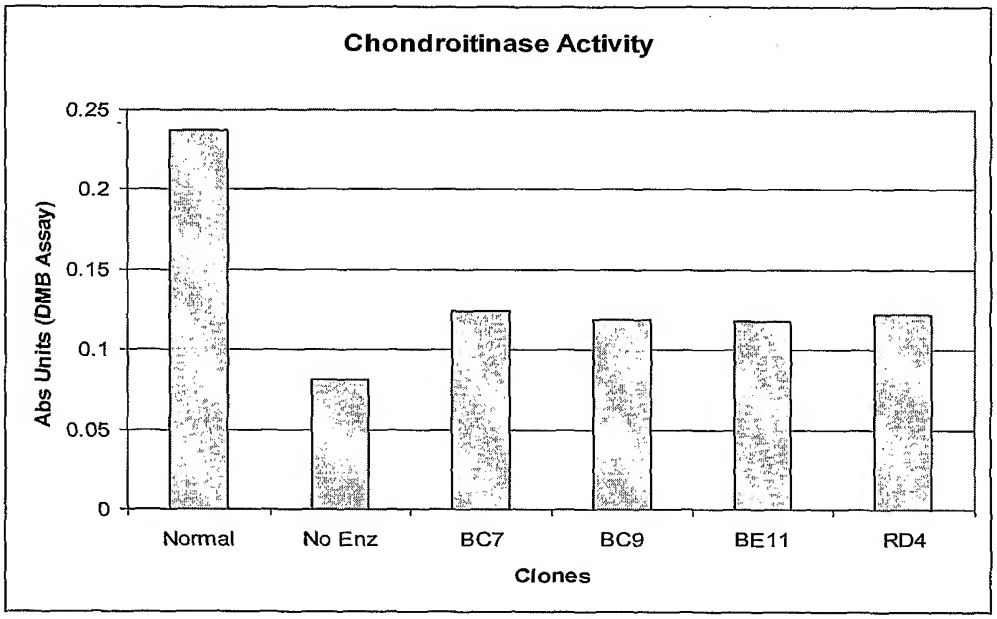
(54) Title: COMPOSITIONS AND METHODS OF USING CHONDROITINASE ABCI MUTANTS



(57) Abstract: One aspect of the present invention relates to mutants of chondroitinase ABCI. Such chondroitinase ABCI mutants exhibit altered chondroitin lyase activity or increased resistance to inactivation from stressors including exposure to UV light or heat. Methods of using chondroitinase ABCI mutant enzymes are also provided.

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A. TITLE: COMPOSITIONS AND METHODS OF USING CHONDROITINASE ABCI MUTANTS

B. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Application No. 60/720,628, filed September 26, 2005 entitled "Compositions and Methods of Using Chondroitinase ABCI Mutants", herein incorporated by reference in its entirety.

C. NOT APPLICABLE

D. NOT APPLICABLE

E. NOT APPLICABLE

F. BACKGROUND

[0002] Proteoglycans, major constituents of the extracellular matrix, are known to be present in large amounts in glial scar tissue and to inhibit recovery following spinal cord injuries (Fawcett & Asher, 1999). Enzymes that are capable of digesting glial scar tissue are an important target for the development of spinal cord injury (SCI) therapeutics. Chondroitinase ABCI (EC 4.2.2.4; cABCI) is a bacterial enzyme that catalyzes the digestion of sulfated chondroitin and dermatan side chains of proteoglycans. This enzyme has been shown to promote functional recovery after spinal cord injury (Bradbury et al., 2002; Caggiano et al., 2005).

[0003] The spinal cord is made up of nerve fibers. Damage to the central nervous system, including the spinal cord, results in a loss of function. Depending upon the type of injury to the central nervous system, the loss of function may manifest itself in loss of sensory, motor or autonomic function or a combination thereof. Sensory functions include the ability to feel sensations, like pain. Motor functions include the ability to voluntarily move your body. Autonomic functions include involuntary body functions, for example the ability to sweat and breathe.

[0004] The most common types of spinal cord injuries (SCI) include contusions (bruising of the spinal cord) and compression injuries (caused by prolonged pressure on the spinal cord). In contusion and compression injuries, a cavity or hole often forms in the center of the spinal cord. Unlike nerve cells, or neurons of the peripheral nervous system (PNS), neurons of the central nervous system (CNS) do not regenerate after injury.

[0005] Spinal cord injury can be characterized by contusion of the neural tissue with a resultant decrease or loss of the ability of nerve tissue to properly transmit nerve impulses. The usual cause is due to an impact injury of some nature, but it may also occur during the manipulation of the spinal cord in certain surgical procedures. After a spinal cord injury in the adult mammal, the inability of axons to regenerate may lead to loss of sensation, loss of motor function and/or loss of autonomic function, as well as permanent paralysis. One reason that neurons fail to regenerate is their inability to traverse the glial scar that develops following a spinal cord injury. The injury-induced lesion will develop glial scarring, which contains extracellular matrix molecules including chondroitin sulfate proteoglycans (CSPGs). CSPGs inhibit nerve tissue growth in vitro and nerve tissue regeneration at CSPGs rich regions *in vivo*.

[0006] A number of molecules, and specified regions thereof, have been implicated in the ability to support the sprouting of neurites from a neuronal cell, a process also referred to as neurite outgrowth. The term neurite refers to both axon and dendrite structures. The process of sprouting neurites is essential in neural development and regeneration, especially after physical injury or disease has damaged neuronal cells. Neurites elongate profusely during development both in the central and peripheral nervous systems of all animal species. This phenomenon pertains to both axons and dendrites.

[0007] Various polypeptides, especially cell adhesion molecules (CAMs), have been known to promote neural cell growth. While early efforts in this area of research concentrated on the adhesion-promoting extracellular matrix protein fibronectin (FN), other polypeptides have also been found to promote neural growth. For example, U.S. Patent No. 5,792,743 discloses novel polypeptides and methods for promoting neural growth in the CNS of a mammal by administering a soluble neural CAM, a fragment thereof, or a Fc-fusion product thereof. U.S. Patent No. 6,313,265 discloses synthetic polypeptides containing the pharmacologically active regions of CAMs that can be used in promoting nerve regeneration and repair in both peripheral nerve injuries as well as lesions in the CNS. While helpful, the use of regenerative proteins alone may not be sufficient to effect repair of a damaged nervous system.

[0008] During approximately the past two decades, knowledge of cell adhesion and migration in extracellular matrices (ECMs) at the molecular level has expanded rapidly. The action of enzymes and other polypeptides which degrade components of the extracellular matrix and basement membranes may facilitate the events of neural repair by a variety of mechanisms, including the release of bound cytokines and by increasing the permeability of

the matrix, thereby enhancing the mobility of mediator molecules, growth factors and chemotactic agents, as well as the cells involved in the healing process. For example, U.S. Patent No. 5,997,863 discloses the use of glycosaminoglycans to manipulate cell proliferation and promote wound healing.

[0009] Components of the inhibitory CSPGs have been identified as the glycosaminoglycans, chondroitin sulfate (CS) and dermatan sulfate (DS). Removal of these inhibitory molecules would allow neurites to regenerate and reinnervate an area after physical injury or disease, as well as to allow for the recovery of sensory, motor and autonomic functions.

[0010] Previous studies have found that chondroitinases can lyse and degrade CSPGs including, CS and DS. One study found that chondroitinase ABC removed glycosaminoglycan (GAG) chains in and around lesioned areas of rat CNS *in vivo*. The degradation of GAGs promoted expression of a growth-associated protein, GAP-43, indicating an increase in the ability of treated cells to regenerate. However, this growth-associated protein is associated with regeneration in peripheral, but not central, nerve injuries.

[0011] Chondroitin sulfates (CS) are sulfated polysaccharides in linear chains of a repeated dissacharides. They range in molecular weight from about 10,000 to over 100,000 Da. Chondroitin sulfate substrates exist in different isomers designated by the appended letters A, B, and C (Hoffman et al., 1958). The repeating units are composed of uronic acid (GlcA or IdoA) and galactosamine, and are called galactosaminoglycans, and are one example of the glycosaminoglycans, typically abbreviated as GAG. Although these GAG chain species have different repeating disaccharide regions, they are covalently bound through the so-called linkage region tetrasaccharide sequence (see below) to the serine residue in the GAG attachment consensus sequence (Glu/Asp-X-Ser-Gly) of respective core proteins. Chondroitin A and C sulfates (ChS-A, ChS-C) are the most abundant GAGs and are found in cartilage, bone and heart valves. Chondroitin B (ChS-B, or, alternatively, dermatan sulfate) is expressed mostly in skin, blood vessels, and heart valves.

[0012] When chondroitinase bacterial preparations were characterized against different chondroitin sulfate (ChS) substrates, a series of distinct chondroitinases were discovered: Chondroitinase AC that degrades mostly chondroitin A (ChA) and chondroitin C (ChC) (Yamagata et al., 1968), Chondroitinase B that degrades chondroitin B (ChB) (Michelacci and Deitrich, 1976), Chondroitinase C that acts mostly on ChC (Michelacci YM & Dietrich CP, 1976) and Chondroitinase ABC exhibits specificity against all three substrates - ChS-A, ChS-B and ChS-C (Yamagata et al., 1968, Michelacci et al., 1987).

G. SUMMARY OF THE INVENTION

[0013] One aspect of the present invention provides mutants of chondroitinase ABCI.

[0014] In preferred embodiments, such chondroitinase ABCI mutants exhibit enhanced activity. In other preferred embodiments, such chondroitinase ABCI mutants exhibit enhanced resistance to inactivation, including invactivation from UV or heat exposure. More preferably, the chondroitinase ABCI mutant enzymes are selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), BF4 (SEQ ID NO:3). In another preferred embodiment, the chondroitinase ABCI mutant enzymes are selected from BC9 (SEQ ID NO:4), BC7 (SEQ ID NO:5), RD4 (SEQ ID NO:6) and BE11 (SEQ ID NO: 7).

[0015] Another embodiment of the present invention is a method of designing mutants of chondroitinase ABCI having altered activity.

[0016] Other embodiments of the present invention relate to methods for promoting neurological functional recovery, including sensory, motor and autonomic function, after central nervous system ("CNS") injury or disease.

[0017] Further embodiments relate to methods of promoting neuronal outgrowth and the use in treating spinal cord injuries and related disorders of the CNS by administering such chondroitinase ABCI mutants.

H. DESCRIPTION OF THE DRAWINGS

[0018] In part, other aspects, features, benefits and advantages of the embodiments of the present invention will be apparent with regard to the following description, appended claims and accompanying drawings where:

[0019] Fig. 1 is a bar graph of the chondroitin lyase activity of wild-type, not-inactivated chondroitinase ABCI (normal), wild-type, inactivated (No Enz) and chondroitinase ABCI mutant enzymes of the present invention following UV exposure.

[0020] Fig 2 is a bar graph of the chondroitin lyase activity of wild-type, not-inactivated chondroitinase ABCI (normal), wild-type, inactivated (No Enz) and chondroitinase ABCI mutant enzymes of the present invention.

I. DETAILED DESCRIPTION

[0021] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular molecules, compositions, methodologies or protocols described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or

embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0022] It must also be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to a "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0023] As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%.

[0024] "Administering" when used in conjunction with a therapeutic means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted. Thus, as used herein, the term "administering", can include, but is not limited to, providing an enzyme into the CNS or onto the target tissue; providing an enzyme systemically to a patient by, e.g., intravenous injection whereby the therapeutic reaches the target tissue; providing an enzyme in the form of the encoding sequence thereof to the target tissue (e.g., by so-called gene-therapy techniques). "Administering" a composition may be accomplished by injection, topical administration, or by either method in combination with other known techniques.

[0025] The term "animal" as used herein includes, but is not limited to, humans and non-human vertebrates such as wild, domestic and farm animals.

[0026] The term "improves" is used to convey that the present invention changes either the appearance, form, characteristics and/or the physical attributes of the target to which it is being provided, applied or administered. The change may be demonstrated by any of the following alone or in combination, including degradation of the CSPGs of the lesioned area of the spinal cord or within the CNS or restoring, in whole or in part, motor, sensory or autonomic function of the mammal.

[0027] The term "inhibiting" includes the administration of a compound of the present invention to prevent the onset of the symptoms, alleviating the symptoms, or eliminating the disease, condition or disorder.

[0028] By "pharmaceutically acceptable", it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0029] The term "recombinant protein" refers to a polypeptide of the present invention which is produced by recombinant DNA techniques, wherein generally, DNA encoding a polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the protein. Moreover, the phrase "derived from", with respect to a recombinant gene, is meant to include within the meaning of "recombinant protein" those proteins having an amino acid sequence of a native protein, or an amino acid sequence similar thereto which is generated by mutations including substitutions and deletions (including truncation) of a naturally occurring form of the protein.

[0030] As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve an unwanted condition or disease of a patient. In part, embodiments of the present invention are directed to the treatment of the central nervous system, such as degradation of the CSPGs of the lesioned area of the spinal cord or within the CNS or restoration, in whole or in part, motor, sensory or autonomic function of the mammal.

[0031] The terms "therapeutically effective amount" or "effective amount", as used herein, may be used interchangeably and refer to an amount of a therapeutic compound component of the present invention. For example, a therapeutically effective amount of a therapeutic compound is a predetermined amount calculated to achieve the desired effect, i.e., to effectively treat an injury to the central nervous system. For example, a therapeutic compound comprising a therapeutically effective amount of chondroitinase which may be purified by a method of the present invention and formulated to provide a stable, active enzyme, is sufficient to degrade the CSPGs of the lesioned area of the spinal cord or an amount sufficient to restore, in whole or in part, motor, sensory or autonomic function of the mammal and may result in a regeneration of neurons in a central nervous system, such as by promoting axonal growth into an injured area.

[0032] The terms "treat," "treated," or "treating" as used herein refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes of this invention, beneficial or

desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

[0033] The term "vector" refers to a vehicle which can transport the nucleic acid molecules. The nucleic acid molecules encoding the chondroitinase polypeptide are covalently linked to the vector nucleic acid. With this aspect of the invention, the vector can be a plasmid, single or double stranded phage, a single or double stranded RNA or DNA viral vector, or artificial chromosome, such as a BAC, PAC, YAC, OR MAC.

[0034] Chondroitinase may be obtained from a microorganism that naturally expresses a chondroitinase; for example, but not limited to, *E. coli*, *Proteus vulgaris or* from the expression of a recombinant protein in a host cell. The host cell can be a prokaryotic cell (such as *E. coli*) or a eukaryotic cell (such as yeast, a mammalian cell or an insect cell).

[0035] The nucleotide sequence of chondroitinase ABCI is set forth as SEQ ID NO. 8 and the amino acid sequence of chondroitinase ABCI is set forth as SEQ ID NO. 9.

[0036] One aspect of the present invention provides mutants of chondroitinase ABCI. In a preferred embodiment, the chondroitinase ABCI mutant enzymes are selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), BF4 (SEQ ID NO:3). In another preferred embodiment, the chondroitinase ABCI mutant enzymes are selected from BC9 (SEQ ID NO:4), BC7 (SEQ ID NO:5), RD4 (SEQ ID NO:6) and BE11 (SEQ ID NO: 7).

[0037] Such enzymes may be formulated into pharmaceutical compositions and formulations. Suitable stable formulations and methods of purification are set forth in co-pending PCT Application No. US2005/017464 filed May 18, 2005 entitled "Methods of Purifying Chondroitinase and Stable Formulations Thereof" herein incorporated by reference in its entirety.

[0038] One aspect of the present invention provides mutants of chondroitinase ABCI. In preferred embodiments, such chondroitinase ABCI mutants exhibit enhanced activity.

[0039] In other preferred embodiments, such chondroitinase ABCI mutants exhibit enhanced resistance to inactivation. More preferably, the chondroitinase ABCI mutant

enzymes are selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), BF4 (SEQ ID NO:3). In another preferred embodiment, the chondroitinase ABCI mutant enzymes are selected from BC9 (SEQ ID NO:4), BC7 (SEQ ID NO:5), RD4 (SEQ ID NO:6) and BE11 (SEQ ID NO: 7).

[0040] Another embodiment of the present invention is a method of designing mutants of chondroitinase ABCI having altered activity. The method comprises altering the nucleotide sequence or amino acid sequence of chondroitinase ABCI, expressing the chondroitinase ABCI in a suitable vector and measuring the activity of the mutant enzyme.

[0041] In a further embodiment, a stable chondroitinase ABCI enzyme is provided. The enzyme may exhibit increased resistance to inactivation under stressed conditions, including exposure to UV light or heat. In a preferred embodiment, the enzyme exhibits increased stability compared to wild-type chondroitinase ABCI enzyme following a challenge by a stress.

[0042] A further embodiment of the present invention is a method of treating central nervous system injuries comprising administering a chondroitinase ABCI mutant enzyme. In preferred embodiments, the chondroitinase ABCI mutant enzyme is administered in a therapeutically effective amount. In a preferred embodiment, the chondroitinase ABCI mutant enzyme is selected from the group consisting of BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), BF4 (SEQ ID NO:3), BC9 (SEQ ID NO:4), BC7 (SEQ ID NO:5), RD4 (SEQ ID NO:6) and BE11 (SEQ ID NO: 7), more preferably, the enzyme is selected from the group consisting of BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), and BF4 (SEQ ID NO:3). Such central nervous system injuries may include, but are not limited to, spinal cord injuries.

[0043] Another embodiment of the present invention is a method promoting neuronal outgrowth comprising administering a chondroitinase ABCI mutant enzyme. In preferred embodiments, the chondroitinase ABCI mutant enzyme is administered in a therapeutically effective amount. In a preferred embodiment, the chondroitinase ABCI mutant enzyme is selected from the group consisting of BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), BF4 (SEQ ID NO:3), BC9 (SEQ ID NO:4), BC7 (SEQ ID NO:5), RD4 (SEQ ID NO:6) and BE11 (SEQ ID NO: 7), more preferably, the enzyme is selected from the group consisting of BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2), and BF4 (SEQ ID NO:3).

[0044] Other embodiments of the present invention relate to methods for promoting neurological functional recovery after central nervous system ("CNS") injury or disease. . In preferred embodiments, the chondroitinase ABCI mutant enzyme is administered in a therapeutically effective amount. In particular, the present invention is directed to a method

of utilizing chondroitinase to promote sensory, motor or autonomic neurological functional recovery following injury in or to the spinal cord. Compositions useful in this method include acceptable formulations of chondroitinase, more particularly sustained release formulations of chondroitinase. The present invention is also directed to a method of promoting neurological functional recovery after a contusion injury to the spinal cord. The most common types of spinal cord injuries (SCI) include contusions (bruising of the spinal cord) and compression injuries (caused by pressure on the spinal cord). In contusion injuries, the most common type of injury, a cavity or hole often forms in the center of the spinal cord.

[0045] The treatments of the present disclosure deliver an effective amount of the mutant or other optional therapeutic agent to the CNS or the injured site of the CNS. Such methods may include optionally administering other chondroitin sulfate proteoglycans, inclulding, but not limited to chondroitinase ABC_{TypeI}, chondroitinase ABC_{TypeII}, chondroitinase AC and chondroitinase B or mammalian enzymes with chondroitinase-like activity such as Hyal1, Hyal2, Hyal3, and Hyal4, preferably to the CNS, and more preferably to the lesions of the injured area of the CNS.

[0046] As is known in the art, chondroitinase polypeptides can be produced by standard biological techniques or by chemical synthesis. For example, a host cell transfected with a nucleic acid vector directing expression of a nucleotide sequence encoding the subject polypeptides can be cultured under appropriate conditions to allow expression of the peptide to occur. The chondroitinase polypeptide may be secreted and isolated and from a mixture of cells and medium containing the recombinant chondroitinase polypeptide. Aspects of the invention described herein provide purification methods wherein the chondroitinase is isolated in a pure form that is more stable and active then those methods currently used.

[0047] Alternatively, the peptide may be retained cytoplasmically by removing the signal peptide sequence from the recombinant chondroitinase gene and the cells harvested, lysed and the protein isolated by the purification methods described herein.

[0048] Chondroitinase may be administered topically, locally or systemically. Topical or local administration is preferable for greater control of application. The chondroitinases, singularly or in combination, can be mixed with an appropriate pharmaceutical carrier prior to administration. Examples of generally used pharmaceutical carriers and additives are conventional diluents, binders, lubricants, coloring agents, disintegrating agents, buffer agents, isotonizing fatty acids, isotonizing agents, preservants, anesthetics, surfactants and the like, and are known to those skilled in the art. Specifically pharmaceutical carriers that may be used are dextran, sucrose, lactose, maltose, xylose,

trehalose, mannitol, xylitol, sorbitol, inositol, serum albumin, gelatin, creatinine, polyethlene glycol, non-ionic surfactants (e.g. polyoxyethylene sorbitan fatty acid esters, polyoxyethylene hardened castor oil, sucrose fatty acid esters, polyoxyethylene polyoxypropylene glycol) and similar compounds. Pharmaceutical carriers may also be used in combination, such as polyethylene glycol and/or sucrose, or polyoxyethylene sorbitan fatty acid esters, polyoxyethylene sorbitan monooleate (20 E. 0.) is particularly preferred.

[0049] A treatment regimen according to the invention may be carried out by a means of administering a mutant chondroitinase ABCI enzyme of the present invention. The treatment regiment may further comprise administering chondroitinase ABCII, chondroitinase AC and chondroitinase B or mammalian enzymes with chondroitinase-like activity such as Hyal1, Hyal2, Hyal3, Hyal4 and PH2O to the lesions of the injured area of the CNS. The mode of administration, the timing of administration and the dosage are carried out such that the functional recovery from impairment of the CNS is enhanced by the promotion of neurite outgrowth.

[0050] The effective amount of chondroitinase can be administered in a single dosage, two dosages or a plurality of dosages. Although it is to be understood that the dosage may be administered at any time, in one embodiment, the dosage is administered within 12 hours after injury, or as soon as is feasible. In another embodiment, the dosage is administered to an injured mammal in one, two or a plurality of dosages; such dosages would be dependant on the severity of the injury and the amount of CSPGs present in the glial scarring. Where a plurality of dosages is administered, they may be delivered on a daily, weekly, or bi-weekly basis. The delivery of the dosages may be by means of catheter or syringe. Alternatively, the treatment can be administered during surgery to allow direct application to the glial scar.

[0051] For example, in some aspects, the invention is directed to a pharmaceutical composition comprising a compound, as defined above, and a pharmaceutically acceptable carrier or diluent, or an effective amount of a pharmaceutical composition comprising a compound as defined above.

[0052] The compounds of the present invention can be administered in the conventional manner by any route where they are active. Administration can be systemic, topical, or oral. For example, administration can be, but is not limited to, parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, oral, buccal, or ocular routes, or intravaginally, by inhalation, by depot injections, or by implants. Thus, modes of administration for the compunds of the present invention (either alone or in combination with

other pharmaceuticals) can be, but are not limited to, sublingual, injectable (including short-acting, depot, implant and pellet forms injected subcutaneously or intramuscularly), or by use of vaginal creams, suppositories, pessaries, vaginal rings, rectal suppositories, intrauterine devices, and transdermal forms such as patches and creams.

[0053] Specific modes of administration will depend on the indication. The selection of the specific route of administration and the dose regimen is to be adjusted or titrated by the clinician according to methods known to the clinician in order to obtain the optimal clinical response. The amount of compound to be administered is that amount which is therapeutically effective. The dosage to be administered will depend on the characteristics of the subject being treated, *e.g.*, the particular animal treated, age, weight, health, types of concurrent treatment, if any, and frequency of treatments, and can be easily determined by one of skill in the art (*e.g.*, by the clinician).

[0054] Pharmaceutical formulations containing the compounds of the present invention and a suitable carrier can be solid dosage forms which include, but are not limited to, tablets, capsules, cachets, pellets, pills, powders and granules; topical dosage forms which include, but are not limited to, solutions, powders, fluid emulsions, fluid suspensions, semi-solids, ointments, pastes, creams, gels and jellies, and foams; and parenteral dosage forms which include, but are not limited to, solutions, suspensions, emulsions, and dry powder; comprising an effective amount of a polymer or copolymer of the present invention. It is also known in the art that the active ingredients can be contained in such formulations with pharmaceutically acceptable diluents, fillers, disintegrants, binders, lubricants, surfactants, hydrophobic vehicles, water soluble vehicles, emulsifiers, buffers, humectants, moisturizers, solubilizers, preservatives and the like. The means and methods for administration are known in the art and an artisan can refer to various pharmacologic references for guidance. For example, *Modern Pharmaceutics*, Banker & Rhodes, Marcel Dekker, Inc. (1979); and *Goodman & Gilman's The Pharmaceutical Basis of Therapeutics*, 6th Edition, MacMillan Publishing Co., New York (1980) can be consulted.

[0055] The compounds of the present invention can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. The compounds can be administered by continuous infusion subcutaneously over a period of about 15 minutes to about 24 hours. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0056] For oral administration, the compounds can be formulated readily by combining these compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by adding a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, but are not limited to, fillers such as sugars, including, but not limited to, lactose, sucrose, mannitol, and sorbitol; cellulose preparations such as, but not limited to, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as, but not limited to, the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0057] Dragee cores can be provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0058] Pharmaceutical preparations which can be used orally include, but are not limited to, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as, *e.g.*, lactose, binders such as, *e.g.*, starches, and/or lubricants such as, *e.g.*, talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. All formulations for oral administration should be in dosages suitable for such administration.

[0059] For buccal administration, the compositions can take the form of, *e.g.*, tablets or lozenges formulated in a conventional manner.

[0060] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*,

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0061] The compounds of the present invention can also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[0062] In addition to the formulations described previously, the compounds of the present invention can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

[0063] Depot injections can be administered at about 1 to about 6 months or longer intervals. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0064] In transdermal administration, the compounds of the present invention, for example, can be applied to a plaster, or can be applied by transdermal, therapeutic systems that are consequently supplied to the organism.

[0065] Pharmaceutical compositions of the compounds also can comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as, *e.g.*, polyethylene glycols.

[0066] The compounds of the present invention can also be administered in combination with other active ingredients, such as, for example, adjuvants, protease inhibitors, or other compatible drugs or compounds where such combination is seen to be desirable or advantageous in achieving the desired effects of the methods described herein.

[0067] The following methods are used to illustrate the various embodiments of the present invention. The methods are exemplary methods and are not meant to limit the invention.

EXAMPLE 1

[0068] The present example illustrates exemplary chondroitinase mutant enzymes of the present invention. All nucleotide and amino acids are indicated as the wild-type and then the mutant version (Wild-type to Mutant).

Mutant ABCI enzyme	Nucleotide sequence	Amino Acid sequence
BC6 (SEQ ID NO. 1)	T1206 to C1206 C1114 to A1114	E403 to G403 W372 to C372
BE7 (SEQ ID NO. 2)	G1925 to T1925 T2226 to G2226	S642 to I642 I742 to M742
BF4 (SEQ ID NO. 3)	T2160 to A2160	N720 to K720e
BC9 (SEQ ID NO. 4)	G1238 to A1238	S413 to N413
BC7 (SEQ ID NO. 5)	A1468 to G1468	K490 to E490
RD4 (SEQ ID NO. 6)	T1661 to A1661	L554 to H554
BE11 (SEQ ID NO. 7)	A1901 to T1901 C1935 to T1935 (in wobble position of codon- does not result in AA change)	D634 to V634

EXAMPLE 2

[0069] The present example illustrates the chondroitin lyase activity of exemplary chondroitinase ABCI mutants according to the present invention following UV exposure. Mutant chondroitinase ABIC genese were generated and transformed into bacteria. Bacteria were grown and the mutagenized chondroitinase expressed. The chondroitinase were then exposed to UV light and their chondroitin lyase activity measured. As depicted in Fig. 1, clone BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2) and BF4 (SEQ ID NO:3) exhibited greater chondroitin lyase activity following exposure to UV light as compared to control.

EXAMPLE 3

[0070] The present example illustrates the chondroitin lyase activity of exemplary chondroitinase ABCI mutants according to the present invention. The chondroitinase lyase activity of Clone BC9, Clone BC7, Clone RD4 and Clone BE11 under normal (i.e., non-stressed) conditions was measured and exhibited decreased activity as compared to control an wild-type chondroitinase ABCI, as depicted in Fig. 2.

[0071] Although the present invention has been described in considerable detail with reference to certain preferred embodiments thereof, other versions are possible. Therefore the spirit and scope of the appended claims should not be limited to the description and the preferred versions contained within this specification.

J. Claims

What is claimed is:

1. A mutant chondroitinase ABCI enzyme selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2) and BF4 (SEQ ID NO:3).

2. The mutant chondroitinase ABCI enzyme of claim 1, wherein said enzyme comprises BC6 (SEQ ID NO:1).

3. The mutant chondroitinase ABCI enzyme of claim 1, wherein said enzyme comprises BE7 (SEQ ID NO:2).

4. The mutant chondroitinase ABCI enzyme of claim 1, wherein said enzyme comprises BF4 (SEQ ID NO:3).

5. A method of treating a central nervous system injury comprising administering a therapeutically effective amount of a mutant chondroitinase ABCI enzyme selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2) and BF4 (SEQ ID NO:3).

6. The method of claim 5, wherein the mutant chondroitinase ABCI enzyme is administered following a contusion injury to the central nervous system.

7. The method of claim 5, wherein the mutant chondroitinase ABCI enzyme is administered following a non-contusion injury to the central nervous system.

8. The method of claim 5, wherein the mutant chondroitinase ABCI enzyme is administered following a spinal cord injury.

9. The method of claim 5, wherein the mutant chondroitinase ABCI enzyme is administered locally.

10. The method of claim 9, wherein the local administration is selected from intrathecal and topical administration.

11. The method of claim 5, wherein said enzyme comprises BC6 (SEQ ID NO:1).

12. The method of claim 5, wherein said enzyme comprises BE7 (SEQ ID NO:2).

13. The method of claim 5, wherein said enzyme comprises BF4 (SEQ ID NO:3).

14. A method of promoting neuronal outgrowth comprising administering a therapeutically effective amount of a mutant chondroitinase ABCI enzyme selected from BC6 (SEQ ID NO:1), BE7 (SEQ ID NO:2) and BF4 (SEQ ID NO:3).

15. The method of claim 14, wherein the mutant chondroitinase ABCI enzyme is administered following a contusion injury to the central nervous system.

16. The method of claim 14, wherein the mutant chondroitinase ABCI enzyme is administered following a non-contusion injury to the central nervous system.

17. The method of claim 14, wherein the mutant chondroitinase ABCI enzyme is administered following a spinal cord injury.

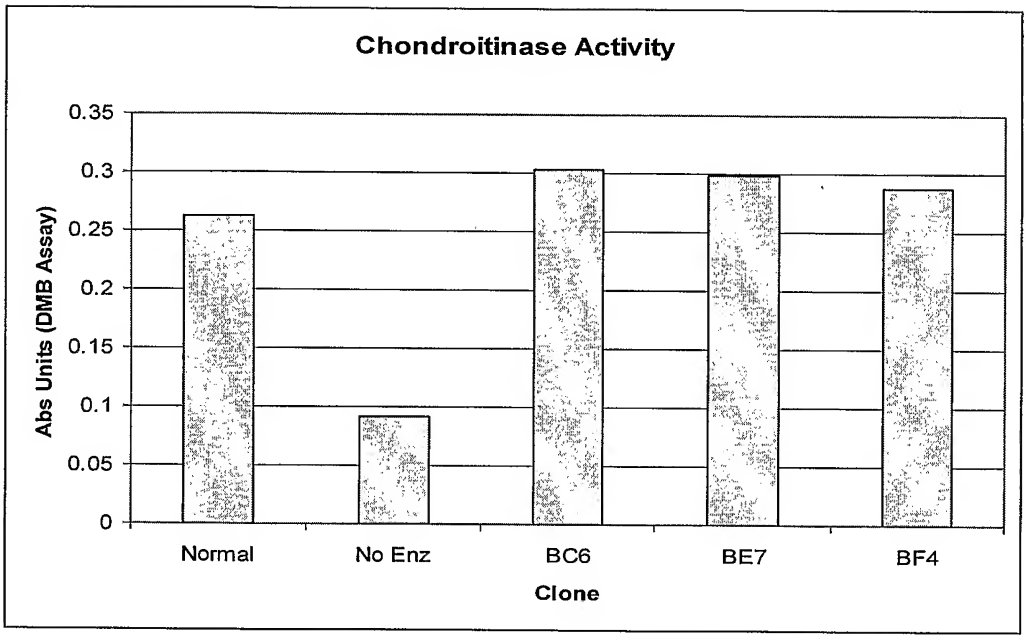
18. The method of claim 14, wherein the mutant chondroitinase ABCI enzyme is administered locally.

19. The method of claim 18, wherein the local administration is selected from intrathecal and topical administration.

20. The method of claim 14, wherein said enzyme comprises BC6 (SEQ ID NO:1).

21. The method of claim 14, wherein said enzyme comprises BE7 (SEQ ID NO:2).

22. The method of claim 14, wherein said enzyme comprises BF4 (SEQ ID NO:3).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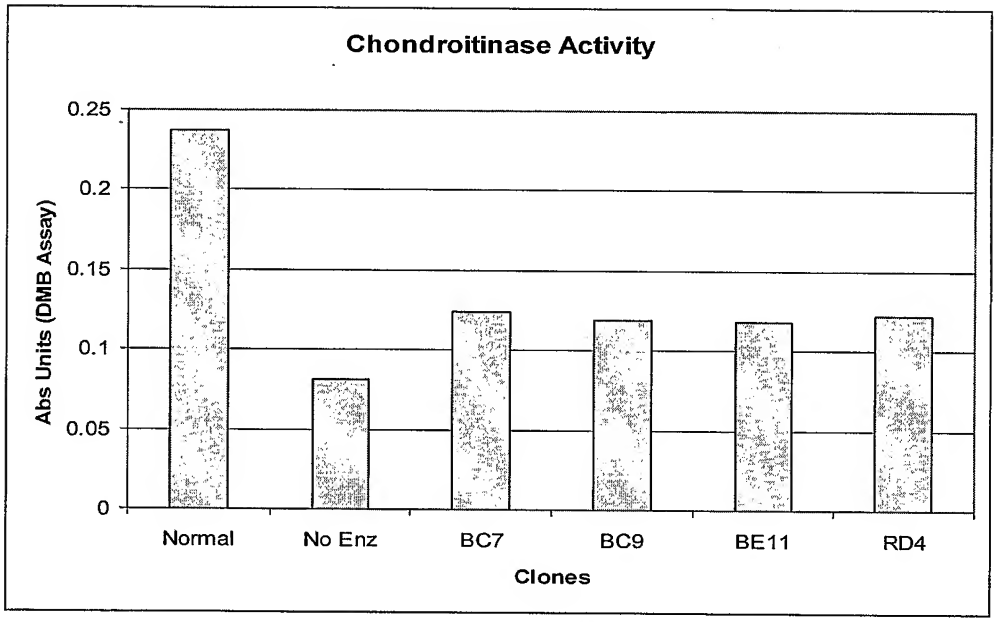
Normal
No Enz
BC6
BE7
BF4
Clone

Fig. 1


Chondroitinase Activity
Abs Units (DMB Assay)
0.25
0.2
0.15
0.1
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Normal
No Enz
BC7
BC9
BE11
RD4
Clones

Fig. 2

ACORDA 3002 SEQUENCES.ST25
SEQUENCE LISTING

<110> ACORDA THERAPEUTICS, INC.
CAGGIANO, ANTHONY O.
IACI, JENNIFER
VECCHIONE, ANDREA
MARKENSOHN, ELIZABETH

<120> COMPOSITIONS AND METHODS OF USING CHONDROITINASE ABCI

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35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
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Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
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Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
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Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
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Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
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180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
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Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
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Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
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Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
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Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
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Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
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Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
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Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
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Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
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Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
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Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
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His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
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Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
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Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
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865 870 875 880

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Thr Gln Asn Glu Ser Thr Ala Ile Phe Gly Glu Thr Ile Thr Pro Ala
580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
595 600 605

His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Asp Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
660 665 670

Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Lys
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
770 775 780

Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
805 810 815

His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
820 825 830

Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
835 840 845

Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
850 855 860

Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
865 870 875 880

Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
885 890 895

Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
900 905 910

Lys Val Asn Lys Pro Ala Ile Val Met Thr His Arg Gln Lys Asp Thr
915 920 925

Leu Ile Val Ser Ala Val Thr Pro Asp Leu Asn Met Thr Arg Gln Lys
930 935 940

Ala Ala Thr Pro Val Thr Ile Asn Val Thr Ile Asn Gly Lys Trp Gln
945 950 955 960

Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
965 970 975

Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
995 1000 1005

Glu Thr Ile Cys Ser Ile Asn Cys Cys Asn Phe Ile Asp Glu Asn
1010 1015 1020

Thr Ile Ala Leu
1025

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Ile Tyr His Phe Ala Gln Asn Asn Pro Leu Ala Asp Phe Ser Ser Asp
20 25 30

Lys Asn Ser Ile Leu Thr Leu Ser Asp Lys Arg Ser Ile Met Gly Asn
35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
50 55 60

Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
65 70 75 80

Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
85 90 95

Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
100 105 110

Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
115 120 125

Arg Thr Val Gly Val Ser Leu Asn Asn Asp Leu Glu Asn Arg Glu Met
130 135 140

Thr Leu Asn Ala Thr Asn Thr Ser Ser Asp Gly Thr Gln Asp Ser Ile
145 150 155 160

Gly Arg Ser Leu Gly Ala Lys Val Asp Ser Ile Arg Phe Lys Ala Pro
165 170 175

Ser Asn Val Ser Gln Gly Glu Ile Tyr Ile Asp Arg Ile Met Phe Ser
180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
195 200 205

Leu Ser Glu Pro Glu Ile Gln Phe His Asn Val Lys Pro Gln Leu Pro
210 215 220

Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
225 230 235 240

Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
260 265 270

Thr Leu Ala Asn Gly Gly Thr Gln Gly Arg His Leu Ile Thr Asp Lys
275 280 285

Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
290 295 300

Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
305 310 315 320

Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
325 330 335

Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
340 345 350

Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
355 360 365

Ser Ser Arg Trp Trp Tyr Ile Ser Thr Leu Leu Met Ser Asp Ala Leu
370 375 380

Lys Glu Ala Asn Leu Gln Thr Gln Val Tyr Asp Ser Leu Leu Trp Tyr
385 390 395 400

Ser Arg Glu Phe Lys Ser Ser Phe Asp Met Lys Val Asn Ala Asp Ser
405 410 415

Ser Asp Leu Asp Tyr Phe Asn Thr Leu Ser Arg Gln His Leu Ala Leu
420 425 430

Leu Leu Leu Glu Pro Asp Asp Gln Lys Arg Ile Asn Leu Val Asn Thr
435 440 445

Phe Ser His Tyr Ile Thr Gly Ala Leu Thr Gln Val Pro Pro Gly Gly
450 455 460

Lys Asp Gly Leu Arg Pro Asp Gly Thr Ala Trp Arg His Glu Gly Asn
465 470 475 480

Tyr Pro Gly Tyr Ser Phe Pro Ala Phe Lys Asn Ala Ser Gln Leu Ile
485 490 495

Tyr Leu Leu Arg Asp Thr Pro Phe Ser Val Gly Glu Ser Gly Trp Asn
500 505 510

Ser Leu Lys Lys Ala Met Val Ser Ala Trp Ile Tyr Ser Asn Pro Glu
515 520 525

Val Gly Leu Pro Leu Ala Gly Arg His Pro Leu Asn Ser Pro Ser Leu
530 535 540

Lys Ser Val Ala Gln Gly Tyr Tyr Trp Leu Ala Met Ser Ala Lys Ser
545 550 555 560

Ser Pro Asp Lys Thr Leu Ala Ser Ile Tyr Leu Ala Ile Ser Asp Lys
565 570 575

Thr Gln Asn Glu Ser Thr Ala Ile Phe Gly Glu Thr Ile Thr Pro Ala
580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
595 600 605

His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Asp Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
660 665 670

Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Asn
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
770 775 780

Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
805 810 815

His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
820 825 830

Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
835 840 845

Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
850 855 860

Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
865 870 875 880

Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
885 890 895

Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
900 905 910

Lys Val Asn Lys Pro Ala Ile Val Met Thr His Arg Gln Lys Asp Thr
915 920 925

Leu Ile Val Ser Ala Val Thr Pro Asp Leu Asn Met Thr Arg Gln Lys
930 935 940

Ala Ala Thr Pro Val Thr Ile Asn Val Thr Ile Asn Gly Lys Trp Gln
945 950 955 960

Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
965 970 975

Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
995 1000 1005

Glu Thr Ile Cys Ser Ile Asn Cys Cys Asn Phe Ile Asp Glu Asn
1010 1015 1020

Thr Ile Ala Leu
1025

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Ala Thr Ser Asn Pro Ala Phe Asp Pro Lys Asn Leu Met Gln Ser Glu
1 5 10 15

Ile Tyr His Phe Ala Gln Asn Asn Pro Leu Ala Asp Phe Ser Ser Asp
20 25 30

Lys Asn Ser Ile Leu Thr Leu Ser Asp Lys Arg Ser Ile Met Gly Asn
35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
50 55 60

Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
65 70 75 80

Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
85 90 95

Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
100 105 110

Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
115 120 125

Arg Thr Val Gly Val Ser Leu Asn Asn Asp Leu Glu Asn Arg Glu Met
130 135 140

Thr Leu Asn Ala Thr Asn Thr Ser Ser Asp Gly Thr Gln Asp Ser Ile
145 150 155 160

Gly Arg Ser Leu Gly Ala Lys Val Asp Ser Ile Arg Phe Lys Ala Pro
165 170 175

Ser Asn Val Ser Gln Gly Glu Ile Tyr Ile Asp Arg Ile Met Phe Ser
180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
195 200 205

Leu Ser Glu Pro Glu Ile Gln Phe His Asn Val Lys Pro Gln Leu Pro
210 215 220

Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
225 230 235 240

Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
260 265 270

Thr Leu Ala Asn Gly Gly Thr Gln Gly Arg His Leu Ile Thr Asp Lys
275 280 285

Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
290 295 300

Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
305 310 315 320

Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
325 330 335

Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
340 345 350

Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
355 360 365

Ser Ser Arg Trp Trp Tyr Ile Ser Thr Leu Leu Met Ser Asp Ala Leu
370 375 380

Lys Glu Ala Asn Leu Gln Thr Gln Val Tyr Asp Ser Leu Leu Trp Tyr
385 390 395 400

Ser Arg Glu Phe Lys Ser Ser Phe Asp Met Lys Val Ser Ala Asp Ser
405 410 415

Ser Asp Leu Asp Tyr Phe Asn Thr Leu Ser Arg Gln His Leu Ala Leu
420 425 430

Leu Leu Leu Glu Pro Asp Asp Gln Lys Arg Ile Asn Leu Val Asn Thr
435 440 445

Phe Ser His Tyr Ile Thr Gly Ala Leu Thr Gln Val Pro Pro Gly Gly
450 455 460

Lys Asp Gly Leu Arg Pro Asp Gly Thr Ala Trp Arg His Glu Gly Asn
465 470 475 480

Tyr Pro Gly Tyr Ser Phe Pro Ala Phe Glu Asn Ala Ser Gln Leu Ile
485 490 495

Tyr Leu Leu Arg Asp Thr Pro Phe Ser Val Gly Glu Ser Gly Trp Asn
500 505 510

Ser Leu Lys Lys Ala Met Val Ser Ala Trp Ile Tyr Ser Asn Pro Glu
515 520 525

Val Gly Leu Pro Leu Ala Gly Arg His Pro Leu Asn Ser Pro Ser Leu
530 535 540

Lys Ser Val Ala Gln Gly Tyr Tyr Trp Leu Ala Met Ser Ala Lys Ser
545 550 555 560

Ser Pro Asp Lys Thr Leu Ala Ser Ile Tyr Leu Ala Ile Ser Asp Lys
565 570 575

Thr Gln Asn Glu Ser Thr Ala Ile Phe Gly Glu Thr Ile Thr Pro Ala
580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
595 600 605

His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Asp Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
660 665 670

Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Asn
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
770 775 780

Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
805 810 815

His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
820 825 830

Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
835 840 845

Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
850 855 860

Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
865 870 875 880

Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
885 890 895

Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
900 905 910

Lys Val Asn Lys Pro Ala Ile Val Met Thr His Arg Gln Lys Asp Thr
915 920 925

Leu Ile Val Ser Ala Val Thr Pro Asp Leu Asn Met Thr Arg Gln Lys
930 935 940

Ala Ala Thr Pro Val Thr Ile Asn Val Thr Ile Asn Gly Lys Trp Gln
945 950 955 960

Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
965 970 975

Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
995 1000 1005

Glu Thr Ile Cys Ser Ile Asn Cys Cys Asn Phe Ile Asp Glu Asn
1010 1015 1020

Thr Ile Ala Leu
1025

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Ala Thr Ser Asn Pro Ala Phe Asp Pro Lys Asn Leu Met Gln Ser Glu
1 5 10 15

Ile Tyr His Phe Ala Gln Asn Asn Pro Leu Ala Asp Phe Ser Ser Asp
20 25 30

Lys Asn Ser Ile Leu Thr Leu Ser Asp Lys Arg Ser Ile Met Gly Asn
35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
50 55 60

Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
65 70 75 80

Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
85 90 95

Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
100 105 110

Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
115 120 125

Arg Thr Val Gly Val Ser Leu Asn Asn Asp Leu Glu Asn Arg Glu Met
130 135 140

Thr Leu Asn Ala Thr Asn Thr Ser Ser Asp Gly Thr Gln Asp Ser Ile
145 150 155 160

Gly Arg Ser Leu Gly Ala Lys Val Asp Ser Ile Arg Phe Lys Ala Pro
165 170 175

Ser Asn Val Ser Gln Gly Glu Ile Tyr Ile Asp Arg Ile Met Phe Ser
180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
195 200 205

Leu Ser Glu Pro Glu Ile Gln Phe His Asn Val Lys Pro Gln Leu Pro
210 215 220

Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
225 230 235 240

Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
260 265 270

Thr Leu Ala Asn Gly Gly Thr Gln Gly Arg His Leu Ile Thr Asp Lys
275 280 285

Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
290 295 300

Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
305 310 315 320

Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
325 330 335

Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
340 345 350

Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
355 360 365

Ser Ser Arg Trp Trp Tyr Ile Ser Thr Leu Leu Met Ser Asp Ala Leu
370 375 380

Lys Glu Ala Asn Leu Gln Thr Gln Val Tyr Asp Ser Leu Leu Trp Tyr
385 390 395 400

Ser Arg Glu Phe Lys Ser Ser Phe Asp Met Lys Val Ser Ala Asp Ser
405 410 415

Ser Asp Leu Asp Tyr Phe Asn Thr Leu Ser Arg Gln His Leu Ala Leu
420 425 430

Leu Leu Leu Glu Pro Asp Asp Gln Lys Arg Ile Asn Leu Val Asn Thr
435 440 445

Phe Ser His Tyr Ile Thr Gly Ala Leu Thr Gln Val Pro Pro Gly Gly
450 455 460

Lys Asp Gly Leu Arg Pro Asp Gly Thr Ala Trp Arg His Glu Gly Asn
465 470 475 480

Tyr Pro Gly Tyr Ser Phe Pro Ala Phe Lys Asn Ala Ser Gln Leu Ile
485 490 495

Tyr Leu Leu Arg Asp Thr Pro Phe Ser Val Gly Glu Ser Gly Trp Asn
500 505 510

Ser Leu Lys Lys Ala Met Val Ser Ala Trp Ile Tyr Ser Asn Pro Glu
515 520 525

Val Gly Leu Pro Leu Ala Gly Arg His Pro Leu Asn Ser Pro Ser Leu
530 535 540

Lys Ser Val Ala Gln Gly Tyr Tyr Trp His Ala Met Ser Ala Lys Ser
545 550 555 560

Ser Pro Asp Lys Thr Leu Ala Ser Ile Tyr Leu Ala Ile Ser Asp Lys
565 570 575

Thr Gln Asn Glu Ser Thr Ala Ile Phe Gly Glu Thr Ile Thr Pro Ala
580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
595 600 605

His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Asp Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
660 665 670

Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Asn
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
770 775 780

Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
805 810 815

His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
820 825 830

Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
835 840 845

Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
850 855 860

Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
865 870 875 880

Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
885 890 895

Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
900 905 910

Lys Val Asn Lys Pro Ala Ile Val Met Thr His Arg Gln Lys Asp Thr
915 920 925

Leu Ile Val Ser Ala Val Thr Pro Asp Leu Asn Met Thr Arg Gln Lys
930 935 940

Ala Ala Thr Pro Val Thr Ile Asn Val Thr Ile Asn Gly Lys Trp Gln
945 950 955 960

Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
965 970 975

Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
995 1000 1005

Glu Thr Ile Cys Ser Ile Asn Cys Cys Asn Phe Ile Asp Glu Asn
1010 1015 1020

Thr Ile Ala Leu
1025

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Ile Tyr His Phe Ala Gln Asn Asn Pro Leu Ala Asp Phe Ser Ser Asp
20 25 30

Lys Asn Ser Ile Leu Thr Leu Ser Asp Lys Arg Ser Ile Met Gly Asn
35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
50 55 60

Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
65 70 75 80

Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
85 90 95

Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
100 105 110

Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
115 120 125

Arg Thr Val Gly Val Ser Leu Asn Asn Asp Leu Glu Asn Arg Glu Met
130 135 140

Thr Leu Asn Ala Thr Asn Thr Ser Ser Asp Gly Thr Gln Asp Ser Ile
145 150 155 160

Gly Arg Ser Leu Gly Ala Lys Val Asp Ser Ile Arg Phe Lys Ala Pro
165 170 175

Ser Asn Val Ser Gln Gly Glu Ile Tyr Ile Asp Arg Ile Met Phe Ser
180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
195 200 205

Leu Ser Glu Pro Glu Ile Gln Phe His Asn Val Lys Pro Gln Leu Pro
210 215 220

Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
225 230 235 240

Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
260 265 270

Thr Leu Ala Asn Gly Gly Thr Gln Gly Arg His Leu Ile Thr Asp Lys
275 280 285

Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
290 295 300

Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
305 310 315 320

Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
325 330 335

Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
340 345 350

Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
355 360 365

Ser Ser Arg Trp Trp Tyr Ile Ser Thr Leu Leu Met Ser Asp Ala Leu
370 375 380

Lys Glu Ala Asn Leu Gln Thr Gln Val Tyr Asp Ser Leu Leu Trp Tyr
385 390 395 400

Ser Arg Glu Phe Lys Ser Ser Phe Asp Met Lys Val Ser Ala Asp Ser
405 410 415

Ser Asp Leu Asp Tyr Phe Asn Thr Leu Ser Arg Gln His Leu Ala Leu
420 425 430

Leu Leu Leu Glu Pro Asp Asp Gln Lys Arg Ile Asn Leu Val Asn Thr
435 440 445

Phe Ser His Tyr Ile Thr Gly Ala Leu Thr Gln Val Pro Pro Gly Gly
450 455 460

Lys Asp Gly Leu Arg Pro Asp Gly Thr Ala Trp Arg His Glu Gly Asn
465 470 475 480

Tyr Pro Gly Tyr Ser Phe Pro Ala Phe Lys Asn Ala Ser Gln Leu Ile
485 490 495

Tyr Leu Leu Arg Asp Thr Pro Phe Ser Val Gly Glu Ser Gly Trp Asn
500 505 510

Ser Leu Lys Lys Ala Met Val Ser Ala Trp Ile Tyr Ser Asn Pro Glu
515 520 525

Val Gly Leu Pro Leu Ala Gly Arg His Pro Leu Asn Ser Pro Ser Leu
530 535 540

Lys Ser Val Ala Gln Gly Tyr Tyr Trp Leu Ala Met Ser Ala Lys Ser
545 550 555 560

Ser Pro Asp Lys Thr Leu Ala Ser Ile Tyr Leu Ala Ile Ser Asp Lys
565 570 575

Thr Gln Asn Glu Ser Thr Ala Ile Phe Gly Glu Thr Ile Thr Pro Ala
580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
595 600 605

His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Val Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
660 665 670

Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Asn
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
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Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
805 810 815

His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
820 825 830

Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
835 840 845

Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
850 855 860

Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
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Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
885 890 895

Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
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Lys Val Asn Lys Pro Ala Ile Val Met Thr His Arg Gln Lys Asp Thr
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Leu Ile Val Ser Ala Val Thr Pro Asp Leu Asn Met Thr Arg Gln Lys
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Ala Ala Thr Pro Val Thr Ile Asn Val Thr Ile Asn Gly Lys Trp Gln
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Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
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Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
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35 40 45

Gln Ser Leu Leu Trp Lys Trp Lys Gly Gly Ser Ser Phe Thr Leu His
50 55 60

Lys Lys Leu Ile Val Pro Thr Asp Lys Glu Ala Ser Lys Ala Trp Gly
65 70 75 80

Arg Ser Ser Thr Pro Val Phe Ser Phe Trp Leu Tyr Asn Glu Lys Pro
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Ile Asp Gly Tyr Leu Thr Ile Asp Phe Gly Glu Lys Leu Ile Ser Thr
100 105 110

Ser Glu Ala Gln Ala Gly Phe Lys Val Lys Leu Asp Phe Thr Gly Trp
115 120 125

Arg Thr Val Gly Val Ser Leu Asn Asn Asp Leu Glu Asn Arg Glu Met
130 135 140

Thr Leu Asn Ala Thr Asn Thr Ser Ser Asp Gly Thr Gln Asp Ser Ile
145 150 155 160

Gly Arg Ser Leu Gly Ala Lys Val Asp Ser Ile Arg Phe Lys Ala Pro
165 170 175

Ser Asn Val Ser Gln Gly Glu Ile Tyr Ile Asp Arg Ile Met Phe Ser
180 185 190

Val Asp Asp Ala Arg Tyr Gln Trp Ser Asp Tyr Gln Val Lys Thr Arg
195 200 205

Leu Ser Glu Pro Glu Ile Gln Phe His Asn Val Lys Pro Gln Leu Pro
210 215 220

Val Thr Pro Glu Asn Leu Ala Ala Ile Asp Leu Ile Arg Gln Arg Leu
225 230 235 240

Ile Asn Glu Phe Val Gly Gly Glu Lys Glu Thr Asn Leu Ala Leu Glu
245 250 255

Glu Asn Ile Ser Lys Leu Lys Ser Asp Phe Asp Ala Leu Asn Thr His
260 265 270

Thr Leu Ala Asn Gly Gly Thr Gln Gly Arg His Leu Ile Thr Asp Lys
275 280 285

Gln Ile Ile Ile Tyr Gln Pro Glu Asn Leu Asn Ser Gln Asp Lys Gln
290 295 300

Leu Phe Asp Asn Tyr Val Ile Leu Gly Asn Tyr Thr Thr Leu Met Phe
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Asn Ile Ser Arg Ala Tyr Val Leu Glu Lys Asp Pro Thr Gln Lys Ala
325 330 335

Gln Leu Lys Gln Met Tyr Leu Leu Met Thr Lys His Leu Leu Asp Gln
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Gly Phe Val Lys Gly Ser Ala Leu Val Thr Thr His His Trp Gly Tyr
355 360 365

Ser Ser Arg Trp Trp Tyr Ile Ser Thr Leu Leu Met Ser Asp Ala Leu
370 375 380

Lys Glu Ala Asn Leu Gln Thr Gln Val Tyr Asp Ser Leu Leu Trp Tyr
385 390 395 400

Ser Arg Glu Phe Lys Ser Ser Phe Asp Met Lys Val Ser Ala Asp Ser
405 410 415

Ser Asp Leu Asp Tyr Phe Asn Thr Leu Ser Arg Gln His Leu Ala Leu
420 425 430

Leu Leu Leu Glu Pro Asp Asp Gln Lys Arg Ile Asn Leu Val Asn Thr
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Phe Ser His Tyr Ile Thr Gly Ala Leu Thr Gln Val Pro Pro Gly Gly
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Lys Asp Gly Leu Arg Pro Asp Gly Thr Ala Trp Arg His Glu Gly Asn
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Tyr Pro Gly Tyr Ser Phe Pro Ala Phe Lys Asn Ala Ser Gln Leu Ile
485 490 495

Tyr Leu Leu Arg Asp Thr Pro Phe Ser Val Gly Glu Ser Gly Trp Asn
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515 520 525

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Ser Pro Asp Lys Thr Leu Ala Ser Ile Tyr Leu Ala Ile Ser Asp Lys
565 570 575

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580 585 590

Ser Leu Pro Gln Gly Phe Tyr Ala Phe Asn Gly Gly Ala Phe Gly Ile
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His Arg Trp Gln Asp Lys Met Val Thr Leu Lys Ala Tyr Asn Thr Asn
610 615 620

Val Trp Ser Ser Glu Ile Tyr Asn Lys Asp Asn Arg Tyr Gly Arg Tyr
625 630 635 640

Gln Ser His Gly Val Ala Gln Ile Val Ser Asn Gly Ser Gln Leu Ser
645 650 655

Gln Gly Tyr Gln Gln Glu Gly Trp Asp Trp Asn Arg Met Pro Gly Ala
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Thr Thr Ile His Leu Pro Leu Lys Asp Leu Asp Ser Pro Lys Pro His
675 680 685

Thr Leu Met Gln Arg Gly Glu Arg Gly Phe Ser Gly Thr Ser Ser Leu
690 695 700

Glu Gly Gln Tyr Gly Met Met Ala Phe Asp Leu Ile Tyr Pro Ala Asn
705 710 715 720

Leu Glu Arg Phe Asp Pro Asn Phe Thr Ala Lys Lys Ser Val Leu Ala
725 730 735

Ala Asp Asn His Leu Ile Phe Ile Gly Ser Asn Ile Asn Ser Ser Asp
740 745 750

Lys Asn Lys Asn Val Glu Thr Thr Leu Phe Gln His Ala Ile Thr Pro
755 760 765

Thr Leu Asn Thr Leu Trp Ile Asn Gly Gln Lys Ile Glu Asn Met Pro
770 775 780

Tyr Gln Thr Thr Leu Gln Gln Gly Asp Trp Leu Ile Asp Ser Asn Gly
785 790 795 800

Asn Gly Tyr Leu Ile Thr Gln Ala Glu Lys Val Asn Val Ser Arg Gln
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His Gln Val Ser Ala Glu Asn Lys Asn Arg Gln Pro Thr Glu Gly Asn
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Phe Ser Ser Ala Trp Ile Asp His Ser Thr Arg Pro Lys Asp Ala Ser
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Tyr Glu Tyr Met Val Phe Leu Asp Ala Thr Pro Glu Lys Met Gly Glu
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Met Ala Gln Lys Phe Arg Glu Asn Asn Gly Leu Tyr Gln Val Leu Arg
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Lys Asp Lys Asp Val His Ile Ile Leu Asp Lys Leu Ser Asn Val Thr
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Gly Tyr Ala Phe Tyr Gln Pro Ala Ser Ile Glu Asp Lys Trp Ile Lys
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Ser Ala Asp Lys Asn Ser Glu Val Lys Tyr Gln Val Ser Gly Asp Asn
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Thr Glu Leu Thr Phe Thr Ser Tyr Phe Gly Ile Pro Gln Glu Ile Lys
980 985 990

Leu Ser Pro Leu Pro Ala Cys Arg Asp Ala Thr His Glu Arg Ala Pro
995 1000 1005

Glu Thr Ile Cys Ser Ile Asn Cys Cys Asn Phe Ile Asp Glu Asn
1010 1015 1020

Thr Ile Ala Leu
1025